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New records of riffle beetles in Vietnam (Coleoptera, Elmidae)

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Abstract. We present the first records of the riffle beetles *Cuspidevia pilosa* Bian, Hu & Tong, 2024 and *Macronevia simplex* (Hinton, 1936), and new records of *Macronychus reticulatus* Čiampor & Kodada, 1998, from central and northern Vietnam. The type locality of *C. pilosa* lies in southern China, while that of *M. reticulatus* is in southern Laos, and *M. simplex* in Western Malaysia. We provide DNA barcoding data (*COI*), along with illustrations of the habitus, aedeagi, and biotopes, as well as distribution maps of these species.

Key words. Biodiversity, distributional range, DNA barcoding, identification, water beetles

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INTRODUCTION

Riffle beetles (Elmidae Curtis, 1830) represent a moderately large family of aquatic dryopoid beetles associated with diverse lotic habitats. The most recent catalogue of Elmidae lists 148 genera and 1,498 species worldwide, along with their geographical distribution information (Jäch et al. 2016). The fauna of Vietnam is diverse and so far with 50 species classified in 14 genera: *Ancyronyx* Erichson, 1847 (3 spp.), *Eonychius* Jäch & Boukal, 1996 (1 sp.), *Graphelmis* Delève, 1968 (10 spp.), *Grouvellinus* Champion, 1923 (4 spp.), *Heterlimnius* Hinton, 1935 (1 sp.), *Indosolus* Bollow, 1940 (1 sp.), *Leptelmis* Sharp, 1888 (5 spp.), *Macronychus* Müller, 1806 (4 spp.), *Ordobrevia* Sanderson, 1953 (3 spp.), *Potamophilinus* Grouvelle, 1896 (2 spp.), *Potamophilus* Germar, 1811 (1 sp.), *Stenelmis* Dufour, 1835 (10 spp.), *Vietelmis* Delève, 1968 (1 sp.), *Zaitzevia* Champion, 1923 (1 sp.), and *Zaitzeviaria* Nomura, 1959 (3 spp.). Most species from Vietnam were described by Delève (1968, 1970), based on material collected by Hungarian entomologists in 1963, and supplemented by data on revised species of Fairmaire (1889), Grouvelle (1889, 1896), and Pic (1923). Later, six *Graphelmis* and three *Macronychus* species were described from Vietnam (Čiampor 2001, 2004, 2005; Čiampor and Kodada 1998, 2004). Jäch (2003) reported *Ancyronyx procerus* Jäch, 1994 and *A. acaroides acaroides* Grouvelle, 1896 from southern Vietnam, while Yoshitomi and Pham (2014) recorded *Ancyronyx yunju* Bian, Guo & Ji, 2012 in northern Vietnam. Thi Thu Ha et al. (2017) added the first record of *Macronychus reticulatus* Čiampor & Kodada, 1998 and listed also genera *Eonychius* and *Indosolus* from Quảng Nam Province in Central Vietnam. The latest new species, *Heterlimnius vietnamensis* Kamite, 2011, was discovered in Thanh Hoá Province in northern Vietnam (Kamite 2011).

In 2022 and 2023, we joined research on water beetles with colleagues from the Vietnam National Museum of Nature and the Vietnam Academy of Science and Technology. We focused on well-preserved biotopes in northern and central Vietnam and sampled in the streams of Thanh Hoá, Vĩnh Phúc, Nghe An provinces, and Hue city. We collected several hundred specimens and identified around 65 species or lineages using *COI* sequences. Here, we provide the first geographic distributional records for two species, and new records for *Macronychus reticulatus* complemented by their DNA barcoding sequences.

METHODS

The material was collected from northern and central Vietnam and sampled in streams of Thanh Hoá, Vĩnh Phúc, Nghe An provinces, and Hue city. For collecting we used a D-frame hand net and applied a multi-habitat scheme to sample significant habitats proportionally according to their presence within a sampling reach. Microhabitats covering less than 5% of the studied area, such as submerged mosses on stones, exposed submerged fine rootlets of shore vegetation, growth of algae and moss in the spray zone of partly

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submerged rock and boulders as well as hygropetric sites were also sampled. During sampling, we positioned the net and disturbed the substrate for a distance that equals the square of the frame width upstream of the net. When taking samples from moss and algae, we placed the net immediately downstream of them while combing the vegetation by hand, enabling the specimens to float into the net. After three to four replicates, the collected material was rinsed twice or three times with stream water, and specimens were sorted directly in the field, and immediately preserved in 96% ethanol. To collect specimens of *Ancyronyx*, *Graphelmis*, and *Macronychus*, we took the submerged wood of physically manageable size ashore and inspected it for the presence of larvae and adults. While the wood pieces dried, we collected the mowing specimens using tweezers.

A portable, compact LED UV blacklight attached to a cloth was used for collecting night-flying adults; we usually collected at well-visible places without vegetation near stream banks.

In the laboratory, genitalia were dissected from specimens and cleared in lactic acid for two days, and temporarily mounted on a microscope slide with a single cavity in Berlese fluid. After examination, male genitalia were mounted in a drop of DMHF on the same card as the respective specimen.

Specimens were examined under a Leica M205C stereomicroscope with fusion optics and diffuse lighting at magnifications up to 160×. Habitus photographs were taken using a Zeiss Axio Zoom.V16 stereomicroscope, diffuse LED lighting, a Canon 5D Mark IV camera, and Zerene Stacker software (Zerene Systems 2024). Male genitalia were photographed using a Zeiss Axio Imager.M2 microscope.

We obtained *COI* (826 bp) sequences from nine *Macronychus reticulatus* specimens, four *Macronevia simplex* specimens, and one *Cuspidevia pilosa* specimen. Sequences are available in the GenBank database, with accession numbers provided in the list of examined materials. DNA was extracted from the whole specimens using the E.Z.N.A. Tissue DNA kit (OMEGA bio-tek, Norcross, Georgia, USA) following the manufacturer's protocol. A fragment of the 3' end of the *COI* gene was amplified using primers C1-J-2183 (Jerry) and TL2-N-3014 (Pat) (Simon et al. 1994). Amplification products were purified using EPPiC Fast (A&A Biotechnology, Gdansk, Poland), and both strands were sequenced by Macrogen Europe Inc. (Amsterdam, The Netherlands). Raw sequences were assembled and edited in Geneious Prime v. 6.1.8 (Dotmatics, Boston, Massachusets, USA). Alignment was performed manually in Mesquite v. 3.81 (Maddison and Maddison 2023). Uncorrected pairwise distances (*p*-distances) were calculated using MEGA v. 11 (Tamura et al. 2021), and haplotype networks were generated with the *geneHapR* package (Zhang et al. 2023) in RStudio v. 2024.12.0 (Posit Software, Boston, Massachusetts, USA).

Morphological terminology follows Kodada et al. (2016) and Lawrence and Ślipiński (2013). Identification was based on Čiampor and Kodada (1998), Jäch and Boukal (1995, 1996), Bian and Ji (2010), and Bian et al. (2024).

Voucher specimens are housed in the Ján Kodada (CKB) collection at the Department of Zoology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia.

The distribution map was created using QGIS v. 3.30 (QGIS.org 2024) and modified using Adobe Illustrator CC 2024 (Adobe Inc. 2024).

RESULTS

Cuspidevia pilosa Bian, Hu & Tong, 2024

Figures 1A, 2C, 3A, E

Cuspidevia pilosus Bian, Hu & Tong 2024: 2.

Note: the incorrect original spelling "*pilosus*" is herewith mandatorily changed to the correct (feminine) gender (ICZN 1999: Article 34.2).

Type locality. Shaoguan, Ruyuan, Nanling, 25°54'44"N, 113°03'02"E, 626 m a.s.l., Guangdong Province, China.

Distribution (Figure 3E). China (Guangdong, Guangxi, Jiangxi) (Bian et al. 2024), Vietnam (**first record**).

Material examined. VIETNAM — Nghe An Province • Con Cuong District; Pu Mat NP; 18°57'48.3"N, 104°48'24.3"E; 260 m a.s.l.; 08.V.2023; J. Kodada & D. Selnekovič leg.; GenBank barcode [PV258739]; 1 ♂, CKB, JK2015.

The specimen was collected from a small, shallow river (Figure 3A), sampled downstream of the Kem Waterfall, where it was found in a mixture of drifted leaves and small pieces of wood detained by the rocks. The river had turbulent water flow, with rapids in the channel, and the substrate contained cobbles, boulders, and a few allochthonous wood and leaf packs.

Identification. *Cuspidevia* Jäch & Boukal, 1995 is initially a monotypic genus, with *C. velaris* Jäch & Boukal, 1995 discovered in China. Subsequently, three additional species have been described: *C. brevis* Bian & Ji, 2010; *C. jaechi* Bian & Ji, 2010; and *C. pilosa*, all distributed in China. This genus is characterized by the follow-

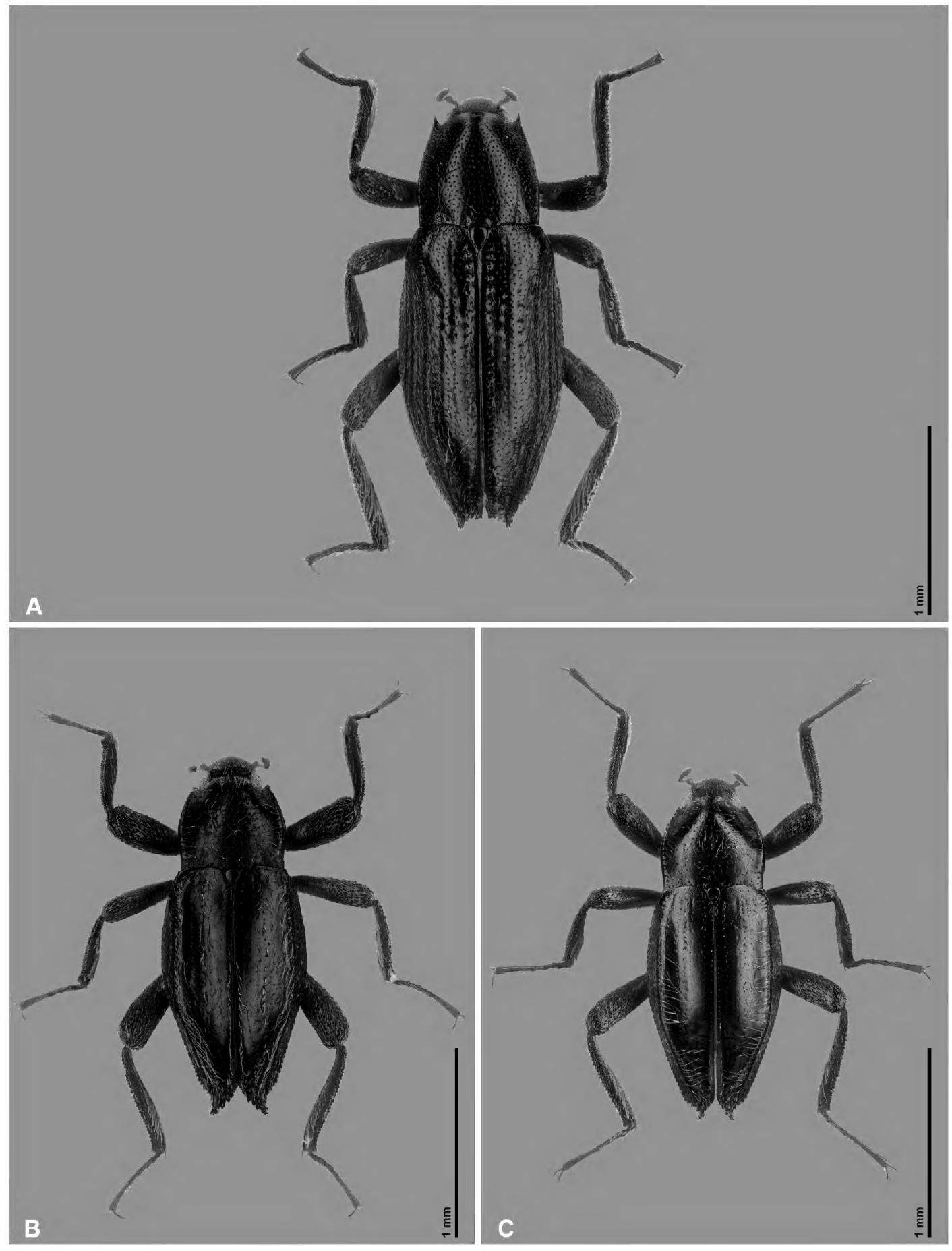

Figure 1. A. *Cuspidevia pilosa* Bian, Hu & Tong, 2024, male from Vietnam, dorsal view. **B.** *Macronevia simplex* (Hinton, 1936), male from Vietnam, dorsal view. **C.** *M. simplex*, male from Malaysia: Pahang, dorsal view.

ing: body elongate, with a scarcely punctate and pubescent, glabrous surface; antennae 8-segmented; anterior angles of pronotum strongly acuminate; pronotum usually with a short, shallow, longitudinal sulcus; elytral apices densely granulate, projecting posteriad; elytral striae almost obsolete, with granulate carina on seventh interval (Jäch and Boukal 1995). *Cuspidevia pilosa* differs from the other three species by having elytral intervals 5, 7, and 8 carinated, pronotum and elytra densely pubescent, and a different aedeagus structure.

Cuspidevia pilosa (habitus as in Figure 1A) is elongate-obovate, moderately convex, with body length ca. 2.7 mm and maximum width across elytra ca. 1.06 mm. Most dorsal surfaces black; ventral portion dark brown; anterior margin of pronotum, tarsi, mouth parts, and antennae brownish.

Pronotum subparallel in basal 0.4, attenuated anteriorly, with acute, distinctly produced anterior angles; posterior angles almost right-angled. Surface irregularly punctured; puncture diameter subequal to facet diameter, distances vary from 0.5–3.0× facet diameter. Moderately long, suberect, yellowish setae arising from punctures (part of setae abraded in specimen examined). Median sulcus absent; sublateral carinae present in basal 0.3 and slightly raised. Elytra broadest in middle, slightly narrowed anteriorly and distinctly attenuated posteriorly, moderately profoundly impressed in anterior third; apices project posteriad and form short tips. Typical strial grooves absent; only first visible and reach anterior 0.4, with large deep punctures (these separated by 1–1.5× puncture diameter); second and third stria indicated from anterior 0.2–0.4 and bear few larger punctures; remaining striae lack grooves, and their punctures small. Intervals smooth and shiny, punctures small and sparsely distributed, with yellowish pubescence. Intervals 5, 7, and 8 carinate;

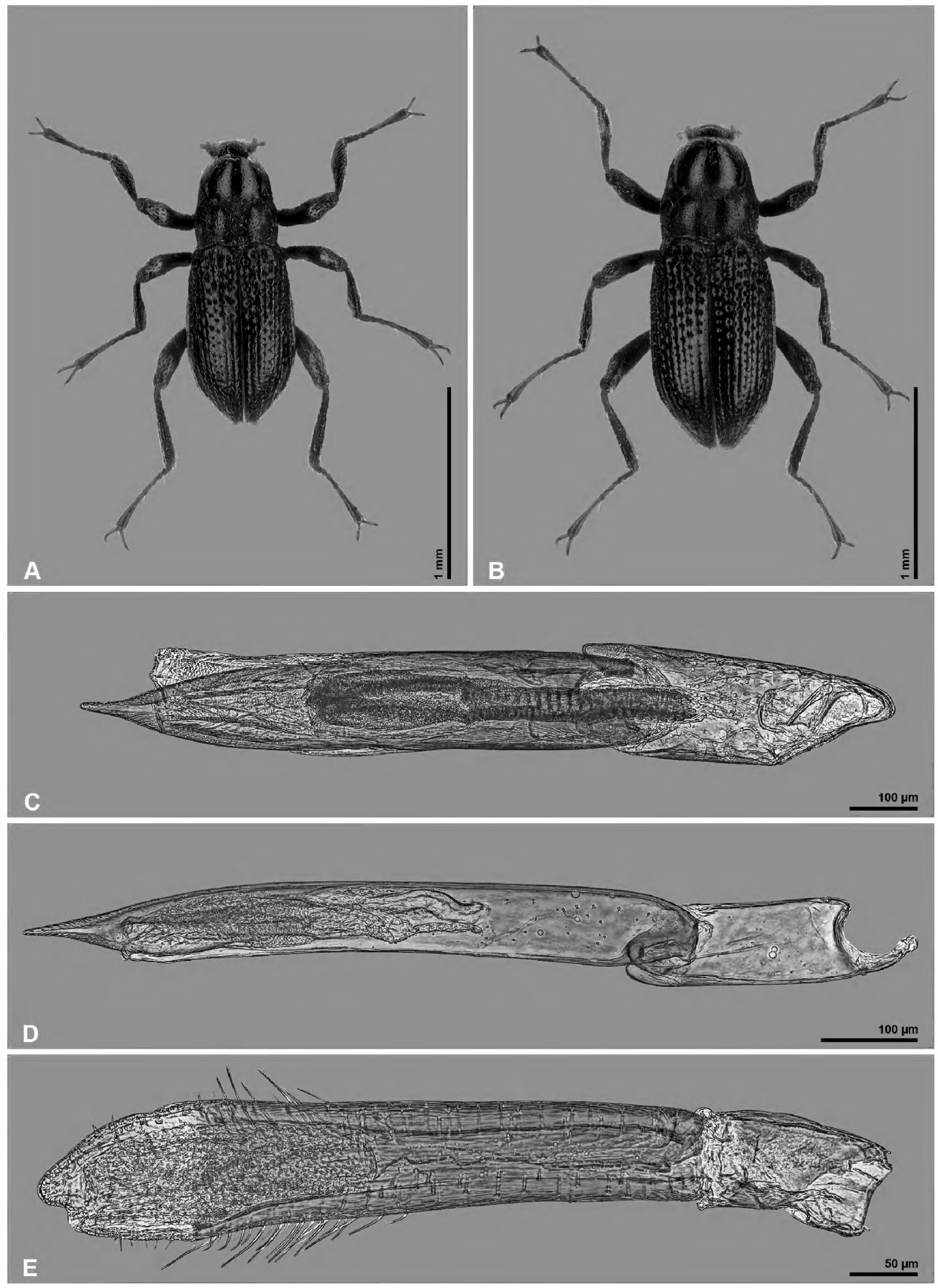

Figure 2. A. *Macronychus reticulatus* Čiampor & Kodada, 1998, male from Vietnam, dorsal view. **B.** *M. reticulatus*, female from Vietnam, dorsal view. **C**. *Cuspidevia pilosa* Bian, Hu & Tong, 2024, aedeagus, ventral view. **D.** *Macronevia simplex* (Hinton, 1936), aedeagus, ventral view. **E.** *M. reticulatus*, aedeagus, ventral view

carinae at 5th and 7th intervals extend from basal 0.1 to apex, carina at 8th interval from basal 0.1 to distal 0.16. Plastron present from interval 5 outwards to elytral margin and intermixed with conspicuous, semierect setae.

Aedeagus (Figure 2C) 1.30 mm long; penis length to phallobase length: 0.85/0.49 mm. Penis gradually narrows apicad from about middle and projects in narrow acute tip; basal portion narrowed; basolateral lobes inserted in apical part of phallobase; surface lacks long hair-like setae. Ventral sac well developed and robust, without fibula and corona. Endophallus (studied only in inverted position) conspicuous and long, with apex inserted into apical third of phallobase. Surface of endophallus in proximal portion with transverse rows of minute spinules, while structures of distal portion extraordinarily complex, with numerous densely arranged, larger, well-sclerotized spines; these spines possibly concealed internal sclerites (everted endophallus position must be studied to identify surface structures). Parameres short and narrow, moderately curved ventrad; one apically with a single moderately long seta, another lacks any seta, each showing two respectively three setal sockets in a similar position. Parameres differentiated within 47–55% of penis length (0.40–0.47 mm), proximally fused with penis wall. Phallobase cylindrical; with ventral, ventrolateral wall and dorsal rim of basal orifice sclerotized; dorsal area membranous; ventro-apical portion emarginated; basal apodeme sclerotized, short and broad, with rounded apex.

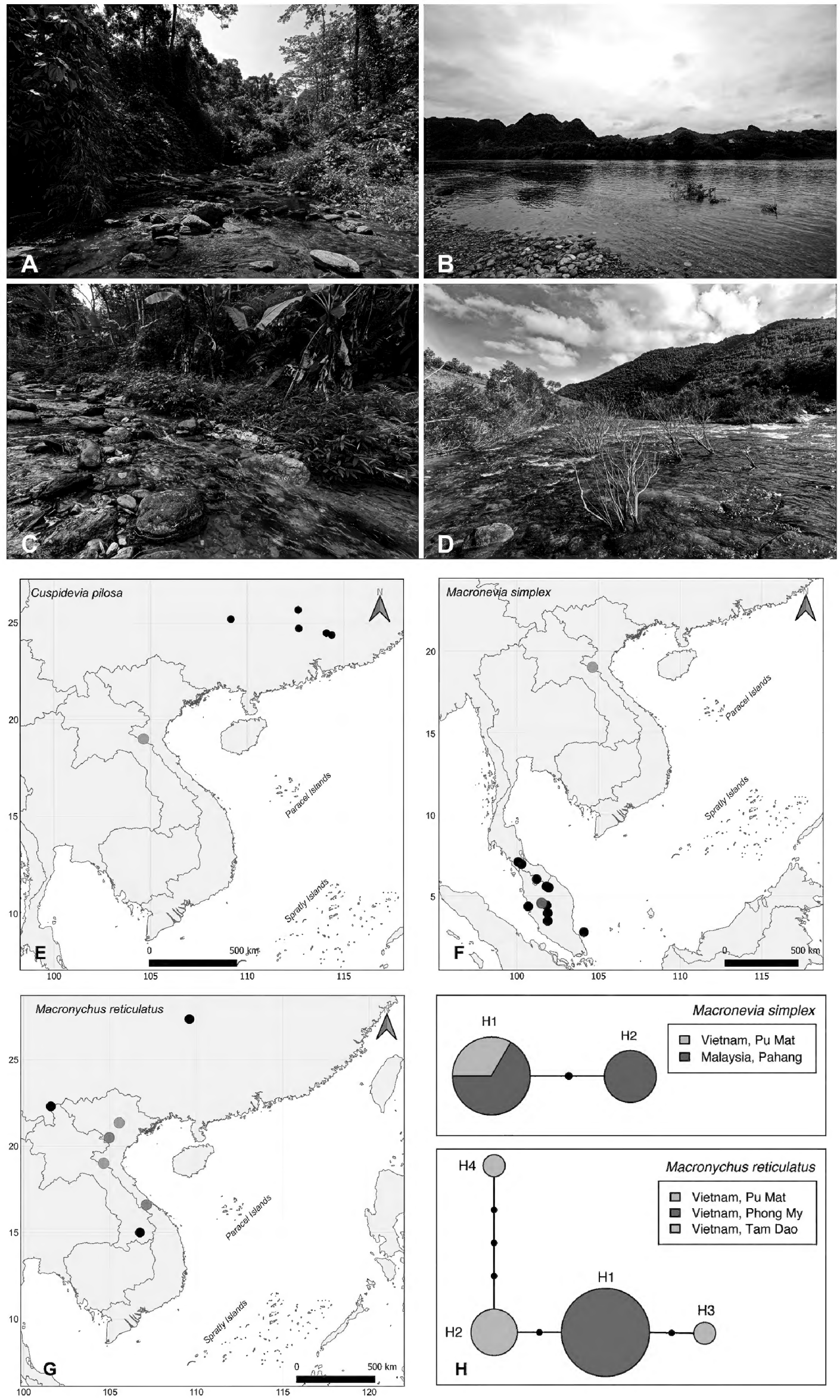

Figure 3. A. Central Vietnam, a small river downstream of the Kem Waterfall flowing in forest, ca. 18°57′48.3″N, 104°48′24.3″E, Pu Mat National Park, the locality of *Cuspidevia pilosa* Bian, Hu & Tong, 2024 and *Macronychus reticulatus* Čiampor & Kodada, 1998. **B.** Central Vietnam, Ca River near 19°03′42.2″N, 104°51′54.7″E, the locality of *Macronevia simplex* (Hinton, 1936). **C.** Northern Vietnam, a stream flowing through small fields and degraded forest near Ko Muong village, ca. 20°28′52.9″N, 105°07′42.4″E, the locality of *M. reticulatus*. **D.** Central Vietnam, Sông ô Lâu River, surrounded by plantations and small fields, ca. 16°31′00.8″N, 107°16′18.7″E, the locality of *M. reticulatus*. **E.** Occurrence records of *Cuspidevia pilosa*. **F.** Occurrence records of *Macronevia simplex*. **G.** Occurrence records of *Macronychus reticulatus*. **H.** Haplotype network of *Macronevia simplex* and *Macronychus reticulatus*. Black circles represent records from previous works (Bian et al. 2024; Čiampor and Kodada 1998; Jäch and Boukal 1996; Shepard and Sites 2016; Thi Thu Ha et al. 2017), other colored circles represent new records from Vietnam.

The male from Vietnam agrees with the original description (Bian et al. 2024); however, it differs in slightly smaller size with narrower elytra and pronotum, but its aedeagus is nearly identical to the published figures.

Macronevia simplex **(Hinton, 1936)**

Figures 1B, C, 2D, 3B, F, H

Macronychus simplex Hinton 1936: 433.

Zaitzevia simplex—Hinton 1940: 113.

Macronevia simplex (Hinton, 1936): 181—comb. nov. by Jäch and Boukal (1996).

Type locality. Pond near Ulu Klang, Selangor, Malaysia.

Distribution (Figure 3F). Malaysia (Kedah, Kelantan, Pahang, Perak, Selangor), Vietnam (**first record**), Thailand (Satun Province, Songkhla Province, Yala Province) (Jäch et al. 2016; Shepard and Sites 2016).

Material examined. VIETNAM — **Nghe An Province** • Con Cuong District, Thanh Dao, Ca River, 19°03′42.2″N, 104°51′54.7″E, 30 m a.s.l.; 09.V.2023; J. Kodada & D. Selnekovič leg.; collected at light; GenBank barcode [PV258738]; 1 ♂, CKB, JK2014.

MALAYSIA — **Pahang** • tributary of Sg. Koyan, 04°16′08.94″N, 101°41′14.88″E; 125 m a.s.l.; 30.VII.2016; FC&ZZ leg.; GenBank barcodes [PV258735, PV258736, PV258737]; 3 ♂, 2 ♀, CKB, JK1379–1383.

The specimen from Vietnam was collected by light trap near the Ca River (Figure 3B).

Identification. *Macronevia* Jäch & Boukal, 1996 is a monotypic genus and belongs to a group of genera which includes *Cuspidevia* Jäch & Boukal, 1995; *Urumaelmis* Satô, 1963, and *Zaitzevia* Champion, 1923. *Macronevia* is characterized by a conspicuous setation of pronotum and elytra and the absence of median and sublateral pronotal grooves (Jäch and Boukal 1996).

Macronevia simplex (Figure 1B, C) with the length of pronotum and elytra 2.0–2.5 mm; maximum width across elytra 0.9–1.0 mm. Color dark brown or almost black; labrum, mouthparts, antennae, tarsi, and anterior margin of pronotum yellowish brown. Body elongate, with scarcely punctate surface; plastron structures on vertex and lateral parts of frons, around eyes, elytra between lateral margin and sublateral carina and on elytral projections, hypomeron, prosternum except for middle, epipleura, lateral parts of meso- and metaventrite, coxae, femora, medial parts of tibiae, and lateral parts of abdominal sternites. Pronotum approximately as wide as long, disc moderately densely covered with semierect setae, lateral parts with adpressed hairs; middle of anterior margin with a group of relatively densely arranged, comparatively long, yellowish setae; sublateral grooves absent or vestigial; disc more or less regularly convex, median groove absent. Elytra elongate, broadest near middle or near anterior third; lateral margin crenulate in apical half; elytral striae more or less obsolete; first to fifth intervals smooth, opaque or glabrous; fifth interval with row of conspicuously long, semi-erect or adpressed, yellowish setae; sutural interval with similar row of distinctly shorter setae; fifth interval conspicuously carinate and crenulate, carina not reaching elytral base; shoulders with very short carina; traces of additional carinae may be present on elytral apex; elytral apices densely granulate, each acuminate and produced posteriad. Legs moderately long; surface of tibiae and femora with small granules; femora with yellowish setae on inner surface; pro- and metafemora clavate.

Aedeagus (Figure 2D) ca. 1.00 mm long, slender; penis length to phallobase length: 0.71/0.29 mm; apex of penis narrowed, acute and inclined moderately dorsad; basal portion of penis narrowed and inserted in apical part of phallobase. Fibula and corona absent; ventral sac well developed and continues into long endophallus (studied only in inverted position); endophallus apex reaches basal third of penis. Endophallus surface in distal portion lacking densely arranged spinules, while proximal portion having numerous acute spines and areas with small, nearly rounded projections; distinct sclerites not detected. Parameres short, differentiated within 55–66% of the penis length (0.40–0.50 mm), their proximal portions fused with penis wall, each parameral apex with two ventral setae. Phallobase cylindrical, with ventral wall and dorsal rim of basal orifice sclerotized; dorsal wall membranous; basal apodeme moderately bent, sclerotized, as long as the phallobase width.

Jäch and Boukal (1996) observed variability of the morphological characters between and within populations, although specimens from the same locality varied to a lesser extent. Examined specimens vary in size and shape of the elytral apices. Apical projections can be broad and curved outwards, as in a male from Vietnam (Figure 1B), narrow and almost straight in a male from Pahang (Figure 1C), or short and almost obsolete with only a short tip projecting. None of the specimens examined had an admedian impression on the pronotal disc, which is present in the holotype, and two specimens studied by Jäch and Boukal (1996). The aedeagus also varies; in a specimen from Vietnam, the apex of the penis is nearly straight (in lateral view). Males from Pahang have moderately narrower apex of the penis. We considered the observed morphological variability intraspecific based on the nearly identical *COI* sequences that detected only two haplotypes with a *p*-distance of 0.13%. Interestingly, haplotype H1 occurs in northern Vietnam as well as in West Malaysia (Figure 3F).

Macronychus reticulatus Čiampor & Kodada, 1998

Figures 2A, B, E, 3A, C, D, G, H

Macronychus reticulatus Čiampor & Kodada 1998: 237.

Type locality. River ca. 40 km east of Muang Paksong, Champasak Province in southern Laos.

Distribution (Figure 3G). Laos, China (Guangdong, Hunan, Yunnan) (Jäch et al. 2016), Vietnam (Thi Thu Ha et al. 2017).

Material examined. VIETNAM — **Thanh Hoá Province** • Bá Thước District, Thành Sơn, Kho Mường; 20°28′52.9″N, 105°07′42.4″E; 415 m a.s.l.; 14.XI.2022; J. Kodada & D. Selnekovič leg.; collected on a small piece

of submerged wood together with *Graphelmis jaechi* Čiampor, 2001 in a small, shallow stream (Figure 3C); CKB, 1♀, JK2000 — **Hue city** • Phong Điền Distr., Phong Mỹ; 16°31′00.8″N, 107°16′18.7″E; 20 m a.s.l., 19.XI.2022; J. Kodada & D. Selnekovič leg.; collected in river ca. 100 m wide, substrate of stones, gravel with sand, and large branches of submerged wood were present near the river margins (Figure 3D); GenBank barcodes [PV258730, PV258731, PV258732, PV258733, PV258734]; CKB, 3 ♀, 1 ♂, JK2361, JK2362, JK2010–2012 — **Vĩnh Phúc Province** • Tam Đảo District, Tam Quan, 21°26′33.0″N, 105°36′30.9″E; 106 m a.s.l.; 26.XI.2022; J. Kodada & D. Selnekovič leg; collected in river ca. 10 m wide with large stones and gravel, submerged wood and roots of shore vegetation were present; GenBank barcode [PV258726]; CKB, 1 ♀, JK1989 — **Nghe An Province** • Con Cuong District, Pu Mat NP; 18°57′36.0″N, 104°48′09.0″E; 305 m a.s.l.; 05–09.V. 2023, J. Kodada & D. Selnekovič leg. (12); collected on submerged wood with three *Graphelmis* spp., and numerous adults and larvae of *Ancyronyx yunyu*; GenBank barcodes [PV258727, PV258727]; CKB, 1 ♀, 1 ♂, JK2122, JK2123; 18°57′48.3″N, 104°48′24.3″E; 260 m a.s.l.; 08.V.2023; J. Kodada & D. Selnekovič leg. (14); collected on submerged wood in a small river ca. 10 m wide (Figure 3A) together with two *Graphelmis* spp. and *Ancyronyx yunju*; GenBank barcode [PV258729]; CKB, 1 ♂, JK2171.

Identification. *Macronychus* represents a small genus currently comprising 12 species. Čiampor and Kodada (1998) published a taxonomic revision of the genus with detailed descriptions and included a key to species identification with numerous illustrations of diagnostic characters for 11 species. The twelfth species, *Macronychus xuhaoi* Jiang & Chen, 2024 was recently described from Xizang in southwestern China (Jiang and Chen 2024). *Macronychus* is characterized within the family Elmidae by the following: antennae 7- segmented; pronotum with two, more or less protruding admedian gibbosities and a translucent anterior margin; third elytral interval tuberculate, ninth carinate; elytral plastron covering the area between lateral margin and ninth interval.

Macronychus reticulatus is a short and narrow species (habitus shown in Figure 2A, B), with a body length of 1.8–2.2 mm and a maximum body width across elytra 0.7–0.9 mm. All specimens examined lack prominent elytral humeri, a characteristic of brachypterous or apterous specimens. Elytra, pronotum, and venter black or dark brown; mouth parts, antennae, palpi, anterior margins of labrum, pronotum, prosternum, tarsi, and claws reddish. Pronotal surface distinctly reticulated in posterior and shiny in anterior half; admedian prebasal gibbosities convex, mesally separated by about 0.25 interocular distance. We found on each gibbosity 2–4 longer, flattened setae arising from moderately larger sockets (in *M. quadrituberculatus* Müller, 1806 setae are more conspicuous and more abundant and form clusters). Setae retained in two specimens examined and probably abraded on others; similar longer and flattened setae bears anterior portion of moderately raised third elytral interval. Scutellar surface reticulated similarly to pronotum. Elytra moderately convex, with highest point near middle, narrow; humeri not prominent; elytral apex emarginate; striae with round, deeply impressed, and rather irregularly spaced macropunctures; strial punctures most prominent on disc and becoming gradually smaller apicad and laterad; intervals narrower than striae, third interval with inconspicuous prebasal tubercle. Legs about as long as body.

Form and structure of aedeagus very characteristic (Figure 2E). Penis, about 3.6–3.9× as long as phallobase, forms well-sclerotized tube from base to apical 0.25; base straight, not inserted into phallobase; sides subparallel with fine constriction in apical 0.33, near apex hyaline (membranous) area continuous with less sclerotized, rounded apex. Lateral setae long and short, positioned along apical 0.4 of penis; shortest setae situated apically and more ventrally, while longest one more dorsally; ventral sac short, confined to apical 0.25 of penis length. Endophallus relatively short, extending to begins of apical third of penis length; sides with two narrow, long sclerotized supporting bands; surface with moderately dense scale-like structures. Free parameres absent. Phallobase cylindrical, with ventral wall and dorsal rim of basal orifice sclerotized; dorsal wall membranous; basal apodeme absent.

Sexual dimorphism. Males are, on average, shorter and narrower than females and show widened protibiae, and the terminal tarsomeres of fore legs bear two ventro-apical setae.

Specimens from Vietnam agree very well with species description and show minimal differences in morphological characters; similar is a minor variability in the *COI* sequences.

In *M. reticulatus*, we identified four distinct haplotypes, with a maximum *p*-distance of 0.48% between haplotypes H1 and H4, corresponding to the significant geographic separation among sampling localities (Figure 3G, H).

DISCUSSION

Cuspidevia pilosa is a recently discovered species from Guangdong, Guangxi, and Jiangxi Provinces of China (Bian et al. 2024). The type material consists of 22 specimens from six localities collected in 2011, 2017, 2020, and 2022. Considering the sampling efforts during the four years, the number of examined specimens is low, suggesting that the species is probably scarce in the sampled locations. A single male specimen from Vietnam was collected from the Pù Mát National Park, a large, forested area in North Central Vietnam. It is a

biosphere reserve with 94,000 ha of natural forest in the core area and a large buffer zone with 100,000 ha. The species seems to be very scarce here, and even the intense sampling for several days in the same stream provided only a single specimen. Although the habitat data of the Chinese records were not specified, the single specimen from Vietnam inhabited a naturally well-preserved biotope.

Macronevia is monotypic, and *M. simplex* was known from southern Thailand and peninsular Malaysia (Jäch et al. 2016; Shepard and Sites 2016). The presented data from Vietnam expands the distributional range to around 3000 km northeast. Jäch and Boukal (1996) discovered and commented on intra- and interpopulation variability of morphological characters. However, despite the pronounced inter-populational variability, they could not work out significant distinguishing features for taxonomic demarcations. We can confirm a very similar variability in apical projections of elytra, body form, as well as in the form of aedeagus in our material examined. However, we detected only two haplotypes with a *p*-distance of 0.13% (difference in 1 nucleotide position), and surprisingly, the haplotype H1 occurs in northern Vietnam as well as West Malaysia (Figure 3F). For clarification of the species variability, it is of course better to study more individuals; however, the molecular results so far indicate that the variability is intraspecific. The factors driving this morphological variability remain unclear.

In Vietnamese specimens of *M. reticulatus*, we identified four distinct haplotypes, with a maximum *p*-distance of 0.48% between haplotypes H1 and H4, corresponding to the greatest geographic separation among sampling localities (Figures 3G, H). Haplotypes H2 and H3 differ only in a single nucleotide position from haplotype H1 (maximum *p*-distance of 0.12%). The variability in *COI* sequences is surprisingly low in comparison with similarly brachypterous specimens of *Ancyronyx pulcherrimus* Kodada, Čiampor & Jäch, 2014 from Sarawak where the documented pattern of nucleotide substitutions and their genetic divergence ranges from 2.5% to 3.7% in sympatric lineages (Kodada et al. 2020). Morphological variability of *M. reticulatus* was observed mainly in the size of specimens; the male genitalia were nearly identical in form and endophallic structures. The distribution in Vietnam is continuous with those in Laos and the southwestern provinces of China. The number of sampled individuals was low, and the species is relatively rare in sampled localities.

Our research activities in Vietnam showed that species diversity is distinctly higher than previously reported, and species with an extensive distributional range exist here. By analysis of sequences of *COI* gene from samples, we detected the presence of about 65 riffle beetle species (or lineages). To identify these species, we need to study the type material, including species described from China, Myanmar, Thailand, and Malaysia.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: JK. Data curation: KG. Funding acquisition: JK, THP. Project administration: JK, KG. Visualization: JK, DS. Writing – original draft: JK. Writing – review and editing: JK, DS, KG, THP. Field works administrations: THP.

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Data availability

All data supporting this study's findings are available in the main text.

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